THE ANTITRYPTIC ACTIVITY OF HUMAN BLOOD SERUM: ITS SIGNIFICANCE AND ITS DIAGNOSTIC VALUE.[1]

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It is not much more than a year ago that Brieger and Trebing announced that a new characteristic of the blood in cases of cancer had been determinend. This new feature consisted in a marked increase in the power of the serum to inhibit the proteolytic activity of solutions of trypsin, and was so constant an accompaniment of cancer as to be present in over 95 per cent. of the cases. Since the publication of their original paper, investigations on this subject have followed each other in rapid succession, so that the literature has come to assume a very goodly volume. As the result of this accumulation of data, it has become increasingly evident that the "antitryptic reaction," as it is called, is not an exclusive characteristic of cancer: it appears under certain other conditions of disease; it marks the change from breast to artificial feeding in infants; it is a striking feature of the onset of labor and the puerperium, as contrasted with pregnancy; in other words, it appears to be a physiological adaptation of widespread significance and value. On the other hand, the fact is not to be gainsaid that the accumulation of evidence has not materially weakened the assumption originally maintained by Brieger and Trebing—the antitryptic reaction is an almost constant accompaniment of cancer, and occurs in a very much smaller proportion of all other diseases. The significance of this association between cancer and this biological change in the character of the serum must be regarded as a matter of some importance, aside from any practical diagnostic application which may attach to it. It is part of the larger problem of the general constitutional influence exercised by the newgrowth upon its host. In a larger view, the physiological value of the reaction, and its general relation to the subject of immunity, is a matter which requires elucidation. In the present paper the subject will be considered from the various points of view which have occupied investigators, and which have been outlined above.

Methods.[2] The methods at present in use for determining the antitryptic value of serum are the fruit of a long process of evolution, the details of which need not here be sketched. There are essentially two methods in common use. The first of these, which was employed by Brieger and Trebing,[3] makes use of plates of coagulated serum as the medium of digestion; on such plates a drop of solution of trypsin within twenty-four hours makes a visible depression or dell. A series of mixtures of the serum under examination, and of a standard solution of trypsin, is prepared, in which a constant quantity of the serum is added to increasing amounts of the ferment. Of each of these mixtures a loopful is transferred to the surface of the plates, which are then incubated at 55° for twenty-four hours. In this manner it is possible to determine in each case just how much of the trypsin solution can be totally inhibited by the standard quantity of serum, and so, to determine the "antitryptic titer" of the serum. The other method, known both as that of Fuld and of Gross,[4] employs a solution of casein as the medium of digestion. The serum in constant, and the trypsin solution in ascending, quantities, are added to a series of test-tubes containing equal amounts of casein in solution. At the end of two hours of incubation, the undigested casein is precipitated in all of the tubes by acidification; note is made of the lowest amount of trypsin which produces complete digestion, and this is taken to indicate the limit of the inhibitive activity of the serum. The results in both methods are expressed in figures, which denote the number of tenths of a cubic centimeter of trypsin inhibited. Through a preliminary determination of the inhibitory limits of normal serum it becomes possible to determine that certain serums have greatly diminished or greatly increased inhibitory power.

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[1] Referat to the American Association for Cancer Research, read at a meeting held in New York City, November 27, 1909.

[2] A more detailed critique of these methods may be found in a paper by the author, Archives of Internal Medicine, 1910, p. 109.

As regards the purely technical details of these methods, it may be said that both of them suffer from rather serious defects. The serum-plate method is open to the same objection which has been made to Mette's method of measuring the peptic activity of the gastric juice by means of the quantitative measurement of the amount of egg albumen which it is capable of digesting. Both egg albumens and the coagulated serums of different animals differ very considerably among themselves in digestibility, so that there is not the required constant basis of comparison (Klineberger and Scholz[5]). Furthermore, the visual appreciation of a minute depression on the surface of a serum plate is a very difficult and inexact procedure. When to these sources of error is added the fact that incubation is necessarily carried out at 55°—certainly far from the optimum for trypsin—and that bacterial contamination is a possible, though not a frequent, element of confusion, it may be understood that the method yields results only approximately accurate. The casein method is certainly far simpler and easier to manipulate. In addition to this, it has the added advantages of greater accuracy in the mixture of reagents, a normal temperature of incubation, and a period of experiment so short as to exclude bacterial contamination; furthermore, the "end-points" of the readings are fairly sharp and accurate. Brieger[6] has objected, with regard to this method, that the classification of serums is more or less inexact; that the results are inconstant; and that the acid may produce a soluble acid albumin, and so obscure the "end-point" of the series of readings. None of these objections, however, is valid in fact; they are all purely theoretical. Numerous other objections have been made to the methods, the most important being that recently advanced by Marcus,[7] who was one of its originators. He states, as is well known to be the fact, that only a portion of the trypsin goes into solution, and hence maintains that there must be a considerable degree of variation in the strength of solutions presumed to be equal. He proposes to obviate this very serious difficulty by means of the use of glycerin extracts of the trypsin, which preserve their strength unaltered for considerable periods of time, and hence may be kept as standard stock solutions. Although apparently valid, this objection does not withstand the test of experiment. As shown by Dr. Feldstein and myself, the strength of a series of solutions of trypsin, made up independently with equal amounts of the ferment and salt solution, is astonishingly constant, and there is no need of the modification suggested by Marcus. The insoluble material is apparently evenly distributed as an inert impurity in the commercial trypsins.

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[3] Berl. klin. Woch., 1[illegible] p. 1041.

[4] Archiv f. exp. Path., 1907, p. 137.

[5] Deut. Archiv f. klin. Med., 1908, p. 310.

The most serious objection which can be made to the method concerns the notation of results. This is apparently very simple. The results are stated in units, which represent the amounts of trypsin inhibited, in tenths of a cubic centimeter of the standard solution used. This mode of representing the results depends on the assumption that the amount of antitrypsin contained by serums is directly proportional to the quantity of trypsin which they are capable of inhibiting. In other words, the method presupposes that a serum of the titer 0.6 is twice as strong as one of 0.3. Neither the method of Brieger nor of Bergmann permits of an experimental tests of this hypothesis, but determinations made with the viscosimeter demonstrate that these relationships are distinctly not so simple as demanded by the theory. If an arithmetical series of solutions of trypsin be prepared, and the necessary inhibitory amount of serum determined for each member of the series, it is found that the quantitative intervals in the higher determination become increasingly larger. Consequently, the proportion indicated by the figures obtained by the serum or casein methods is entirely incorrect. Nevertheless, the relative antitryptic strength of the serums is correct, at least from a quantitative standpoint, and it is fair to accept the grouping of serums obtained by these methods as approximately accurate.

[6] Berl. klin. Woch., 1908, 1415, in report of discussion.
[7] Ibid., 1909, p. 156.

The viscosity method[8] previously referred to depends on the fact that the amount of digestion produced by trypsin in gelatin may be measured by determining the alteration in the viscosity of the latter medium. The effect of serum in controlling the activity of the trypsin is, of coure, very simply determined. The method offers certain very distinct advantages over those previously employed. Whereas, by the serum or casein methods, it is possible to make only one determination, namely, either the point of complete inhibition or of total digestion, the viscosimeter determines the degree of inhibition at any moment of time. The method, therefore, substitutes the use of a single mixture for that of a series, which is a very considerable gain in simplicity of technique. Moreover, the method is incomparably more flexible than those previously used, and permits of the determination of a large number of factors otherwise inaccessible to investigation.

The results obtained by the use of the two earlier methods have been strikingly concordant. Indeed, in comparing the findings yielded in a series of cases by both the serum and the casein methods, I found that the data were practically interchangeable. Brieger originally asserted that about 95 per cent. of the cases of cancer evinced a marked increase in the antitryptic value of their serum.[9] He subsequently found that in a large number of other conditions, including both acute infections and chronic wasting diseases, the same phenomenon could be observed, and he consequently concluded that all diseases associated with intense destruction of body protein produced this characteristic alteration in the plasma of the blood. Hence, although he continued to affirm the diagnostic value of the method in all cases of undetermined newgrowth, he reached the conclusion that in general all conditions of "cachexia"—using that term in the broad metabolic sense of wasting disease, either acute or chronic—were competent to produce it. Further investigation has, in general, given ample confirmation to these conclusions, if the term cachexia is interpreted in the peculiar sense in which it was used by Brieger. All observers are agreed that the great majority of cases of cancer give evidence of increased antitryptic value in the serum. In some series the percentage of positive results in cases of cancer ranges as high as 95 per cent.; in others as low as 70 per cent. It is, however, very frequently found in the acute infections, such as pneumonia, typhoid fever, sepsis, and polyarticular rheumatism; in chronic infections, notably tuberculosis; in diabetes and severe anemias; and in Graves' disease almost constantly. These data amply demonstrate that the change in the serum is not to be regarded as a characteristic biological response to the presence of newgrowths. They indicate that it is an evidence of pathological derangement of much wider distribution. Furthermore, it seems misleading to consider the reaction as characteristic of conditions of cachexia, in view of the fact that this term must be extended so as to include a large number of conditions which can by no possibility be classified as cachectic. The general condition of nutrition of patients whose serum yields the reaction is frequently excellent, and could never be understood as cachectic. The theory of causation involved in the term "Kachexie Reaktion" will be subsequently discussed, but the term itself should certainly be allowed to fall into disuse

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[8] Since the presentation of this paper, a preliminary report on the viscosity method has appeared, Feldstein and Weil, Proc. Soc. Exp. Biol. and Medicine, February, 1910.

[9] Berl. klin. Woch., 1908, pp. 1349 and 2260.

Not only under pathological conditions, however, does this reaction occur. It has been found to accompany and characterize certain processes which may be called physiological, although they denote a certain alteration in the normal course of metabolism. Its occurrence in the blood of infants has been investigated, and it has been found that such infants as are being nourished at the breast never display an increased antitryptic content of the blood (Reuss[10]). On the other hand, with the inauguration of artificial feeding, the reaction at once becomes prominent. In pregnancy it has been found that no antitryptic reaction occurs, but with the onset of labor it makes its appearance, and persists through the puerperium (Becker[11]).

It is evident that these findings may be discussed either from the standpoint of their diagnostic value, or as a biological phenomenon of purely theoretical interest. Diagnostically, the opinion of the various authors who have worked upon this problem is strikingly in accord. As a general diagnostic method, the increase in the antitryptic index occurs in too many conditions to have the value of a specific symptom. On the other hand, the absence of the antitryptic reaction in the blood may be taken generally as arguing against the existence of cancer. In the presence of a neoplasm of doubtful character, a positive reaction, in the absence of complicating conditions, notably tuberculosis, argues with a strong degree of probability in favor of the diagnosis of malignancy (Roche,[12] Hort,[13] Braunstein,[14] Bayly[15]).

The method has apparently stood the test of clinical experience, and has proved to be of distinct value when applied rigidly within the prescribed limits. As regards other conditions, Meyer[16] asserts that the reaction occurs with such regularity in cases of Graves' disease, that it may be relied upon in the diagnosis of the numerous obscure and abortive forms of the disease known as *formes frustes.* It has been claimed by Wiens[17] that the strength of the reaction has

[10] Wiener klin. Woch., 1909, p. 1171.

[11] Münch. med. Woch., 1909, p. 1363.

[12] Archives of Internal Medicine, 1909, p. 249.

[13] Brit. Med. Jour., 1909, p. 966.

[14] Deut. med. Woch., 1909, p. 573.

[15] Brit. Med. Jour., 1909, p. 1220. (Bayly measured digestion by electroconductivity.)

[16] Berl. klin. Woch., 1909, p. 1064.

[17] Deut. Archiv f. klin. Med., 1909, p. 62.

marked prognostic value in the acute infections, but this view is certainly erroneous.

Looked at as a biological phenomenon, the reaction suggests many problems, and has given rise to a considerable amount of research. The chemical nature of the antitrypsin, its character as a specific "immune body," its relationship to artificially produced antitrypsin, and the causes of its production, are subjects which demand elucidation, if the physiological significance and the pathological import of the reaction are to find an explanation.

The chemical basis of the antitryptic reaction has not been satisfactorily determined. It was originally asserted by Glaessner[18] that the antitrypsin was associated with the euglobulin fraction of the serum, which is that part of the serum globulins least soluble in water, and roughly corresponds to the fraction which comes down in dialyzing or on adding acetic acid to the diluted serum (Hedin[19]). Cathcart,[20] on the other hand, asserts that the globulins do not possess antitryptic action, but that this is characteristic of the albumin fraction, that is, the fraction precipitated between half and full saturation with ammonium sulphate. Schwarz[21] has reached the conclusion that the antitryptic fraction of the serum exists in the form of a lipoid. He found that he could inactivate antitryptic serums by washing out the lipoids with ether. Such serums could be reactivated by the addition of lecithin. Lecithin in salt solution emulsion, if added to serum, exercised considerable tryptic inhibition; if the mixtures were kept at 65° for one hour, this inhibitory activity was markedly enhanced, indicating that the inhibitory substance is a lipoid-albumin compound. Furthermore, he found by analysis that increase in the antitryptic titer of a serum was constantly associated with an increase in the amount of ether-soluble substances which it contained. These conclusions substantiate the earlier findings of Pribram. Interesting as are these data, they fail to support the contention that antitrypsin is a lipoid substance. It is well known, for example, that lipoids are essential to the activation of cobra venom in the production of hemolysis; nevertheless, it would be erroneous to consider the lipoid substance as the active hemolysin. Lipoids have been shown (Bang[22]) to play an analogous auxiliary role in many processes of immunity, while the essential factor, the active agent, is a protein. It is conservative to maintain this position with reference to antitrypsin, admitting meanwhile the possible importance of lipoids as subsidiary factors.

Is the so-called antitryptic action of the serum dependent on the presence of an "immune body," or is it an accidental property of the serum? This question is really of fundamental importance, though not at all easy to answer. It was found by Vernon[23] that egg albumin in solution inhibits the digestive action of trypsin very actively. But it is a still more striking fact that charcoal has been shown to act as an antitryptic agent, in a manner very similar to serum. The amount of inhibition is proportional to the quantity of charcoal, to the time of interaction, and to the temperature, just as it is in the case of serum. In fact, "the action of charcoal was found to agree with that of the tryptic antibody in all respects tried, and therefore the neutralizing effect, in all probability, is brought about in the same way in both cases" (Hedin[24]). It is perfectly apparent that neither egg albumen nor charcoal can in reality contain a true antitrypsin, and that, therefore, the effect observed is simply an accidental phenomenon. It is true of all human serums that they very notably inhibit the hemolytic effect of saponin; it would, however, be entirely unjustifiable to argue from this fact to the existence of an "antisaponin." These theoretical objections to the assumption of an "antitrypsin" in the serum have, unfortunately, not received recognition in the recent literature. On the other hand, it must be admitted that certain observations argue strongly in favor of a true antitrypsin, as against a general property of serum albumin, in the interpretation of tryptic inhibition. Chief among these is the alleged specificity of the antitryptic action. Specificity is, as is well understood, one of the most striking characteristics of all forms of antibody, and its absence may well be interpreted as a powerful argument in the negative. Eisner,[25] as the result of a series of tests made with the same serums against rennet, pepsin, emulsin, and cobra lipase, arrived at the conclusion that serum does not exhibit the properties of a general ferment inhibitor, but that it possesses a special and characteristic affinity for trypsin. This observation seems to indicate the existence of a specific antibody, a true antitrypsin. Glaessner has also, on insufficient evidence (Cathcart), asserted that the antitrypsin of serum is most active against the trypsin of the same species, and is somewhat specific even for various animal trypsins. The facts do not, however, appear to bear out these contentions. It has been possible in our laboratory, by means of the viscosimeter, to demonstrate that all human serums inhibit papain, which is a vegetable proteolytic ferment, in a constant ratio to the degree with which they inhibit trypsin. It seems, therefore, impossible to accept the specificity of the antitrypsin of the serum. The antitryptic function is exercised by an albuminous substance, thermolabile, indeed, like the true antibodies, but differing essentially from these in the lack of specificity. In view of this fact, and of certain other differences, the argument (Meyer[26]) in favor of a true antibody as the basis of this function of the serum loses very materially in credibility.

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[18] Hofmeister's Beiträge, 1903, iv, 79.

[19] Jour. Physiol., 1903, p. 193.

[20] Ibid., 1904, xxxi, 496.

[21] Wien. klin. Woch., 1909, p. 1151.

[22] Ergebnisse d. Physiologie, 1909, p. 463.

[23] Jour. Physiol., 1904, p. 346.

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[24] Biochemical Journal, 1906, p. 484.
[25] Ztschr. f. Immunitätsforschung, 1909, ii, 650.
[26] Berl. klin. Woch., 1909, p. 2139.

This conclusion makes it very much simpler to dispose of the much debated problem as to the identity of the normal antitrypsin of the serum with that produced artificially in animals by the injection of trypsin. All antiferments hitherto produced by this method have been found to be characteristically specific. Thus, by the injection of rennet, Morgenroth[27] succeeded in producing an antirennin which powerfully inhibited the action of the injected ferment, but had no influence on vegetable rennet. The entire subject of antiferments is, however, in such a condition of confusion that it is almost impossible to draw any very definite conclusions. Achalme[28] produced an active antitrypsin by the injection of trypsin, but Landsteiner failed to reproduce this result. In our own laboratory, the injection of trypsin into guinea-pigs has been uniformly without effect. The most striking experiments are those recently reported from the Pasteur Institute on the result of the injection of pepsin and of papain. It has been shown by Cantacuzène and Jonescu[29] that when rabbits are immunized to pepsin by the injection of increasing doses, the serum responds by the production of an antibody capable of fixing complement, but possesses no increased antiferment action. Similarly, Pozerski[30] has shown that the serum of animals immunized to papain contains a specific precipitin, and an antibody which fixes complement in a characteristic fashion; but this very immunized serum is just as easily digested by the ferment as is normal serum. The natural antitrypsin, so-called, differs, therefore, in many important particulars from the antibody artificially produced by the injection of ferments into animals, and this fact constitutes an additional argument for regarding it as something essentially different from a true antibody.

The conception of antitrypsin as an antibody has, however, dominated practically all the theories which have hitherto been advanced in the attempt to explain it. In spite of the fact that it cannot properly be so regarded, these theories do not necessarily forfeit their validity. It is perfectly reasonable to assume that the serum may respond to a given stimulus by means of a protective mechanism which does not answer to the criteria characteristic of antibodies and amboceptors. It has generally, and very naturally, been assumed that the presence of antitrypsin in the serum is evidence of an effort on the part of the organism to protect itself against self-digestion. If this be the case, then a tryptic ferment should, theoretically, be present in the serum, and this has actually been demonstrated to be the fact by Hedin and by Delezenne;[31] it is, therefore, an important matter to determine its source of supply.

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[27] Centralbl. f. Bakteriologie, 1899, p. 349.

[28] Annales de l'Institut Pasteur, 1901, xv, 736.

[29] Compt.-rend., de la Soc. de Biol. 1909, p. 53.

[30] Annales de l'Institut Pasteur, 1909, p. 205.

[31] Compt.-rend. de la Soc. de Biol., 1903, lv, 132.

There are four of such sources theoretically conceivable at present, namely, the pancreas, the leukocytes, the organs, and the new-growths. Each of these has had, and has, its champions, and each requires consideration. The pancreas, as a source of supply of trypsin, is a very obvious suggestion. Ambard[32] suggests that the antitryptic reaction is so marked in cases of gastric carcinoma, because this condition is associated with compensatory overactivity of the pancreas. There is, however, no evidence that the pancreatic trypsin is absorbed from the intestine, and circulates in the serum. Moreover, the explanation fails to explain the increase in Graves' disease, or the acute infections. The polynuclear leukocytes, as is well known, contain an active proteolytic ferment in most respects identical with trypsin. It has been urged that the constant disintegration of leukocytes must necessarily free a considerable amount of this ferment in the serum, and Hedin is of the opinion that the tryptic ferment which he succeeded in isolating from the serum actually represents the remnant of the intraleukocytic ferment. The view that the antitryptic reaction of serum is the manifestation of a response to the excessive disintegration of leukocytes has been urged and defended by Jochmann,[33] Wiens,[34] Weins and Schlecht,[35] Bittorf,[36] Landois,[37] and many others. As a result of the study of a large number of pathological conditions in which the differential leukocyte curve has been carefully plotted, and the antitryptic strength of the serum also has been repeatedly determined, it appears that there are certain definite relationships between these two factors. It has been quite satisfactorily demonstrated that with the onset of an infection the antitryptic index of the serum falls, and that with the progress of the infection it gradually rises again to the level of the normal, and then passes well beyond this to a highly increased index (Landois). Wiens, and Wiens and Schlecht, have shown that these fluctuations in the antitryptic index are accurately foreshadowed by variations in the leukocyte count, but only in so far as the polynuclear leukocytes determine these variations. The mononuclear cells do not play any role in influencing the index, and it is, therefore, of importance in the understanding of the interrelationship of these phenomena that the differential count should invariably be made. The explanation of these relationships is based on the well-known fact of the trypsin content of the polynuclear cells. With the onset of acute infections there is an immediate and rapid increase in the number of circulating polynuclear leukocytes. The inevitable destruction of a certain proportion of these cells frees an excessive amount of trypsin, which at once neutralizes all the available antitrypsin in the serum. Consequently, the antitryptic index falls well below normal, and may even disappear. This is the so-called negative phase of the antitryptic curve. The excess of trypsin in the blood, however, stimulates the production, or the mobilization, of fresh quantities of antitrypsin, which, in accordance with Ehrlich's interpretation of Weigert's laws of regeneration, are well in excess of the amount of trypsin to be neutralized. Consequently, there is a rapid rise in the antitryptic index. This is the so-called positive phase of the curve. A regulating mechanism of some kind tends, however, to keep the amount of antitrypsin in circulation only a little in excess of the trypsin, and this gives the value of the normal index. Fluctuations of this character, in which the leukocyte count and the curve of the antitryptic index pursue a parallel course, constitute a very striking feature of all infectious conditions. Wiens went so far as to assert that in such conditions a constantly increased antitryptic index was an omen of ill import, and augured the paralysis of the mechanism of defence, specifically the polynuclear leukocytes. Thaller[38] reached similar conclusions with reference to puerperal sepsis. In this belief they were unquestionably in error, inasmuch as the index is dependent quite as much on the amount of antitrypsin liberated by the body as on the leukocytes which represent the reaction to the disease. Indeed, the majority of acute infections, whether the body is in the ascendant or not, are associated with an increase in the antitryptic index. In addition to these clinical observations, there is ample experimental evidence (Miller[39]) that the injection of leukocytes, or of leukocyte extracts, into animals is followed, after a preliminary fall, by a marked rise in the antitryptic index. It may be seen from the preceding analysis that cases of myelogenous leukemia would not necessarily be associated with any notable variation in the antitryptic index, inasmuch as the regulatory mechanism maintains the index at its constant normal level. Jochmann, however, asserts that sudden myelocyte crises may so flood the blood with trypsin that the serum actually assumes digestive power, in place of its normal inhibitory function.

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[32] Sem. méd., 1908, p. 532.

[33] Münch. med. Woch., 1908, lv, 728; Hofmeister's Beiträge, 1908, p. 449.

[34] Deut. Arch. f. klin. Med., 1907, p. 456; Münch. med. Woch., 1907, p. 2637; Centralbl. f. Inn. Med., 1908, p. 773.

[35] Deut. Archiv f. klin. Med., 1909, p. 44.

[36] Ibid., 1907, xci, p. 212.

[37] Berl. klin. Woch., 1909, p. 440.

The preceding theory, interesting as it is, does not fully explain the phenomena. There are many conditions, such as diabetes, Graves' disease, and so forth, in which there is no increased production of leukocytes, yet the antitryptic index is constantly increased. If the leukocyte curve be admitted to afford a satisfactory explanation of the index in infectious conditions, there still remains a considerable number of conditions in which some other explanation must be discovered. In addition to the pancreas and the polynuclear leukocytes, there is another possible source of trypsin in the body, namely, the cells of the tissues. It has now been abundantly shown that many, if not all, of the tissues contain proteolytic ferments, which are competent to break up these tissues outside of the body into a much simpler group of compounds. This process is known as autolysis, and the ferments in question are called autolytic ferments. Although they are actively proteolytic, they appear to differ in certain particulars from true trypsin. Thus, the end-products indicate that an ereptic ferment is almost certainly at work (Vernon[40]). Furthermore, it has been asserted by Jacoby[41] that these ferments are adapted specifically to the proteolysis only of the organs in which they occur, a characteristic which, *if well founded* (Beebe),[42] would sharply differentiate them from true trypsin. In spite of these objections, there are certain facts which indicate their possible relationship with the antitryptic phenomenon of the serum. In the first place, it is known that the injection of tissue other than the pancreas may induce a rise in the antitryptic index. Further, serum exercises an anti-autolytic (Baer and Loeb[43]), just as it does an antitryptic, power. Finally, it has been shown by Shaffer and Buxton,[44] and others, that glycerin extracts of the various organs, including the muscles, are capable of displaying marked proteolytic powers, when tested, for example, upon milk agar plates. The difficulty in all observations of this kind consists in excluding the leukocytes themselves. If it must be admitted that the conditions of experimentation have not yet permitted a final decision as to the character of the ferments contained in the organs, the fact still remains that the tissues do contain a proteolytic ferment, probably very similar to trypsin. In view of this fact, the theory has been advanced that the destruction of body protein from any cause would tend to free the proteolytic ferments contained in the cells, and that the somatic reaction would liberate an excess of antitrypsin in the serum. This theory, it will be seen, is simply an expansion of the leukocyte theory, inasmuch as the leukocytes may be considered as a type of cell distinguished by their increased content of proteolytic ferments. There can, indeed, be no question that it offers an explanation for the increased index in a large number of diseases for which the leukocyte theory is entirely inadequate, such as the marasmus of infants (Lust[45]). This includes not only conditions such as Graves' disease, diabetes, and chronic tuberculosis, but certain acute infections, such as typhoid fever, not associated with leukocytosis. There is, however, one very weak point in this theory, in spite of the fact that it apparently harmonizes with clinical conditions, and this is the unwarranted assumption that heightened protein metabolism is necessarily associated with the liberation

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[38] Berl. klin. Woch., 1909, p. 850.

[39] Zntralbl. f. Chir., 1909, p. 75.

[40] Intracellular Enzymes, 1909.

[41] Ztschr. f. Physiol. Chem., 1901, vol. xxxiii.

[42] Boston Med. and Surg. Jour., 1907.

[43] Arch. f. exp. Path., 1905, p. 1; 1906, p. 68.

[44] Jour. Med. Research, 1905.

[45] Deut. med. Woch., 1909, p. 1901.

of intracellular ferments. The advocates of the theory have devoted a great deal of effort to the support of this assumption. It has been asserted (Fürst) that starvation, with its accompanying cellular destruction, raises the index, but this again has been denied (Meyer). The effect of cellular poisons, such as pilocarpin, phosphorus, and potassium cyanide, has been tested, but without the expected result in raising the index. The kidneys have been tied off, and allowed to necrose *in situ*, but in spite of the presumptive absorption of the cellular constituents including ferments, no rise in the antitryptic index was observed. It must be admitted, therefore, that experimental data fail to give any support to this theory.

The increase of the index in cancer is attributed to the same cause, namely, the liberation of the intracellular ferments, which are well known to be very active in cancerous growths (Bamberg[16]). With the frequent tendency to necrosis in tumors, even if only in microscopic areas, there would seem to be abundant opportunity for the absorption of ferments. But here, again, the absence of evidence that such a process actually does occur is a fundamental flaw in the theory.

To sum up, the origin of the hypothetical trypsin which is supposed to act as a stimulant for the production of antitrypsin, or, technically speaking, as antigen, is as yet undetermined. It may, conceivably, arise in the pancreas, or in the leukocytes, or in the tissue cells, or in the newgrowths, or in each one of these, under varying circumstances, but actual evidence that it does so arise is at the present time an absolute necessity for the establishment of the theory. The very first essential is to determine whether or not the trypsin, or proteolytic ferment of the blood is increased, in accordance with the assumption of the hypothesis, in the conditions which give rise to an increase in the antitryptic index. At the present time, no method seems to be available for this purpose.

THE WASSERMANN AND NOGUCHI COMPLEMENT-FIXATION TEST IN LEPROSY.[1]

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The first to obtain a positive Wassermann reaction in a case of leprosy was Eitner[2] in 1906. A similar report was made by Weichsel-

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[16] Berl. klin. Woch., 1908, pp. 1396 and 1673.

[1] Read at a meeting of the Medical Society, of the State of New York, January 24, 1910.

[2] Ueber den Nachweis von Antikörpern im Serum eines Leprakranken mittels Komplementablenkung, Wien, klin. Woch., 1906, No. 15, p. 1555.